ANTI-CANCER COMPOUNDS AND METHODS RELATED THERETO

The present application claims priority to US Provisional Patent application serial Number 60/097,210, filed August 20, 1998 and US Provisional Patent application serial number 60/141,169, filed June 25, 1999.

Field of the Invention

The present invention relates generally to the field of cancer treatments, as well as to the field of peptide and non-peptide pharmaceutical compounds.

BACKGROUND OF THE INVENTION

Many lung and prostate cancers, of which small cell lung cancer (SCLC) is a prime example, have a neuroendocrine phenotype, and their growth is stimulated by neuropeptides. Antagonists of several peptides (e.g. bradykinin, substance P, bombesin) have been used in experimental treatment of models of SCLC in animals. Among the most potent of the peptides examined thus far, crosslinked dimers of certain bradykinin antagonist peptides have been efficacious both *in vitro* and *in vivo* against strains of SCLC and other tumors (Chan et al., *Immunopharmacology* 33: 201-204, 1996; Stewart et al., *Can. J. Physiol. Pharmacol.* 75: 719-724, 1997; Stewart et al., US Patent Application 5,849,863, issued12/15/98). Prostate cancers show a similar neuroendocrine phenotype and are susceptible to neuropeptide antagonists.

SUMMARY OF THE INVENTION

The present invention provides anti-cancer agents (ACA) comprised of a range of novel amino acid derivatives and small peptides having the ability to inhibit growth of SCLC and certain other tumor cell lines (such as non-small cell lung cancer (NSCLC) and prostate cancer) in standard *in vitro* tests, as well as certain monomeric peptides that show inhibition of tumor growth *in vivo*. Certain of the peptides have a general structural relationship to carboxy-terminal

fragments of bradykinin antagonists, but the non-peptides show no such general relationship. Monomers, dimers, trimers, tetramers, pentamers and cyclized analogs of the novel molecules are described. The new compounds are tested for bradykinin antagonist activity in standard assays, but there is no apparent relationship between bradykinin antagonist activity and cytolytic potency. All of the molecules described possess both hydrophobic (usually aromatic) and basic groups in their structures. Without being held to one particular theory, it appears that the compounds function by stimulation of cell death (apoptosis) in the tumor cells.

The present invention also provides compounds and methods for inhibiting cancer by administering to a subject afflicted with cancer (ie. of the lung or prostate) a therapeutically effective amount of one or more of the compounds herein described.

In general, the anti-cancer compounds are described by the formula:

$$[ACA]_n - X \qquad \text{[Formula I]}$$

wherein X is a linker having 2-5 functional groups or is absent, n = 1-5, and ACA is selected from the group consisting of Formula II, Formula III, Formula IV, Formula V, and Formula VI. Other compounds described herein are defined by the Formula VII. The specifics regarding structure are enumerated in the Detailed Description, Examples and Claims. Certain physical charateristics are enumerated in the Examples as well as the Detailed Description, Examples and Claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows inhibition of growth *in vivo* of SCLC strain SHP-77 by B10054.

Figure 2 shows inhibition of growth *in vivo* of NSCLC strain A-549 by M620.

Figure 3 shows inhibition of growth *in vivo* of SCLC strain SHP-77 by B9430.

Figure 4 shows inhibition of growth *in vivo* of SCLC strain SHP-77 by B10238

Figure 5 shows inhibition of growth *in vivo* of SCLC strain SHP-77 by M570, both as the trifluoroacetate salt and as the hydrochloride salt.

Figure 6 shows inhibition of growth *in vivo* of SCLC strain SHP-77 by M822.

Figure 7 shows inhibition of growth *in vivo* of SCLC strain SHP-77 by M638.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a range of monomeric, dimeric, trimeric, tetrameric, pentameric and cyclic small peptides and peptide mimics that are effective as anti-cancer agents.

In general, the anti-cancer agents (ACA) are described by the formula:

$$[ACA]_n - X \qquad \text{[Formula I]}$$

wherein X is a linker group having 2-5 functional groups or is absent, n = 1, and ACA is selected from the group consisting of Formula II, Formula III, Formula IV, Formula V, and Formula VI, as described herein. Other compounds described herein are defined by the Formula VII, as described herein.

X can be any linking group which does not interfere with the inhibitory activity of the monomer-linker or oligomerized product using *bis*-imido-esters, *bis*-maleimidoalkanes such as *bis*-maleimidohexane, dicarboxylic acids, tricarboxylic acids, tetracarboxylic acids and multi carboxylic acids. Alkane groups may be substituted with alkyl, amino, carboxyl, halogen, hydroxy, mercapto or methoxy groups. Aminoalkyl, aromatic or cycloalkyl polycarboxylic acids, heterocyclic polycarboxylic acids, carboxylic anhydrides and polyoxyethylene linkers may also be used. For C-terminal crosslinking, X may be any diamino or polyamino alkane, cycloalkane, aromatic, heterocyclic diamine, polyamine or other substituted chelating agent (for example: diethylenetriaminepentaacetic dianhydride, ethylenediaminetetraacetic dianhydride, etc.). Polyamino-polycarboxylic acids may also be used to make heteromers (such as ethylenediamine-N,N'-diacetic acid, etc.).

The linkage may be at the N-terminal or the C-terminal or at any position of the ACA sequence through side-chain functional groups. The linker may have any chain length.

For dimers, there is a correspondence between linker length and cytotoxicity. Alkyl chains of 8 carbons or more are preferred, with those of 8 to 18 carbons being most preferred. Examples of preferred dimer linkers for the α-amino at the N-terminal or for a basic side-chain

group at any position of ACA include ADA, BTAC, DDD, DDS, DTP, EGS, EOPC, HDD, HFG, PFS, SBEC, SUB, SUIM and TDIM. For dimerization through the C-terminal carboxyl or any side-chain carboxyl in ACA, the preferred linkers include DDA, DEA, EDA, EDP and HAD. Any di-functional molecule can be used.

For trimers, linkers for basic groups include BTAC, BTC, CHTC, CTAC and TREN-$(Suc)_3$; for carboxyl groups, TREN. Any tri-functional molecule can be used.

For tetramers, linkers can be BAPTA, CPTA, EDTA, EGTA, ETTA, or any tetra-functional molecule.

For pentamers, the linker can be DTPA or any pentameric functional molecule. Compounds formed by ACA and a linker X may be homo or hetero multimers.

[Formula II] comprises:

$$R\text{-}A^{-1}\text{-}B^{0}\text{-}C^{1}\text{-}D^{2}\text{-}E^{3}\text{-}F^{4}\text{-}G^{5}\text{-}H^{6}\text{-}I^{7}\text{-}J^{8}\text{-}K^{9}$$

wherein R, A, B, C, D, E, F, G, H, I, J, and K are selected from the following or may be absent, and wherein K is Arg or an Arg derivative:

R	A -1	B 0	C 1	D 2	E 3	F 4	G 5	H 6	I 7	J 8	K 9
Absent or	Absent or	Absent or	Absent or	Absent Or	Absent or	Absent or	Absent or	Absent or	Absent or	Absent or	Absent or
3,3DP	DmK	Apc	ApC	MeP	Hyp	Ava	Add	Arg	2Nal		Arg
Aaa	Lys	Arg	Arg	Nig	Pro	BAla	Aud	Gly	DCpG	2Nal	Arg(H)
Ac	Lys(εFlu)	DArg	DmK	NMF		Dpr	CpG	Pac	DF5F	2Nal-NH_2	Arg-CH_2OH
Aca	NiK	DLys	NiK	Pro		Eac	DDMF	Pac	DIgl	3,4F2F	Arg-NH_2
BApg	PzO	DmK	NiO			Gly	DMF	Ser	DPFF	3Pal	Arg(NO_2)
Cca		DniK	PaF				Eac	Thr	DPhe	Ac6c	Arg-OMe
Cin		DpaF	PzO				Igl		DTic	Aic	DArg
Dca		DPzK					Lys		Gly	Ana	DArg-NH_2
Dcg		DPzO					Pac		*m*ABz	Apb	DArg(NO_2)
Dhq		Lys					Phe		*p*ABz	Apb	

Dmac	NiK		Thi	Pac	Atpc
Dpa	PaF			PaF(Dcg)	Bip
F5bz	PzO			*p*Amb	Cmp
F5c	DArg-(NO$_2$)	Arg-(NO$_2$)			CpG
F5pa					DhPhe
Gun					Dpr(Fbz)
Hxa					Dpr(Paa)
Mca					F5F
Mcg					F5F-NH$_2$
Moti					Hphe
Pcc					Ica
Ppa					Igl
Pya					Igl-NH$_2$
Saa					Ileu
Ste					Lys(CH$_3$)$_3$
Tfmc					Lys(F5bz)
					M*apa*
					MBC
					MFF
					Nc6G
					Nc7G
					NMF
					OBS
					OBT
					OBY
					OC2Y
					Oic
					Oic-NH$_2$
					*P*AB*z*
					Pac
					PaF(F5c)
					PaF(Fbz)
					PaF(Mcg)
					PaF(Ppa)
					PaF(Sin)
					*p*Amb
					*p*APa
					PCF

PdF
PFF
PFF-NH_2
Phe
PNF
Thi
Tic
Trp
Trx
Tyr

5.

[Formula III] comprises:

$$R\text{-}A^{1}\text{-}B^{2}\text{-}C^{3}\text{-}D^{4}\text{-}E^{5}\text{-}F^{6}$$

wherein R, A, B, C, D, E, and F are selected from the following or may be absent, and wherein F is not Arg or an Arg derivative:

R	A 1	B 2	C 3	D 4	E 5	F 6
Absent or	Absent or	Absent or	Absent or	Absent or	Absent or	Absent or
2,2Dp	DArg	Arg	Add	2Nal	1Nal	2Nal
3,3Dp	DArg(NO_2)		Aud	3Pal	2Nal	3Pal
Aaa			Ava	Arg	2Nap	ABza
Ac			Eac	Arg(Tos)	3Pal	ABza
Aca			Lys	Atcp	Apa	Ama
Boc			Pac	D2Nal	Arg	Ampy
Chc				DArg	Arg-NH_2	Ampz
Cin				DArg(Tos)	Asp	Apa
Ctim				DF5F	Atc	Api
Dca				DIgl	Atcp	Aptp
Dcg				DPFF	Bip	Aqd
Dhq				Eac	BtA	Aqu
Dmac				F5F	Cys(Meb)	Arg(H)
Dns				Gly	Cys(SO3H)	Arg-CH_2OH
Dpa				His	D2Nal	Arg-NH_2
F5c				Igl	DArg	Arg-OMe
F5pa				*m*ABz	DArg-NH_2	Asp
F5po				OC2Y	F5F	Asp(Aqu)
Gbc				Pac	Glu	Atcp
Gun				PFF	Gly	Atmp
Hxa					Igl	AtmpO
Mcg					Inp	Atpm
Mse					Iqa	Cyh

Pya		*m*ABz	Dmab
Seb		MC2Y	Dmm
Sin		N-Dmb-Tyr(Bz)-OMe	Dmp
Sul		OC2Y	Dpea
Tfmc		OCIY	Dpma
Tha		Oic	Dpr(Dcg-2-Nap)
		*p*ABz	Ecap
		PaF(Mes)	F5F-NH_2
		PFF	GaP
		Tic	*m*A_2Bz
		*t*Leu	*m*A_2Bz(Dcg)
		Trp	*m*A_2Bz(Gun)
		Try	*m*ABz
		Try(Bzl)	Mapp
		Tyr	Matp
		Arg(NO_2)	MatpO
			*p*ABz
			PaF
			PaF(Dcg)
			PaF(Mcg)
			PaF-NH_2
			PFF-NH_2
			PgF
			PzO
			Sud
			Thm
			Thm
			Tpac
			Tpac
			Tyr(Bz)OMe

[Formula IV] comprises:

A^0-B^1-C^2-D^3-E^4-F^5-G^6-H^7-I^8-J^9-K^{10}-L^{11}

wherein A, B, C, D, E, F, G, H, I, J, K and L are selected from the following or may be absent:

A	B	C	D	E	F	G	H	I	J	K	L
0	1	2	3	4	5	6	7	8	9	10	11
Absent or DArg	Absent or Arg DArg	Absent or Pro	Absent or Lys	Absent or Pro	Absent or DTrp	Absent or Gln DNMF	Absent or DTrp	Absent or Phe	Absent or DTrp	Absent or Leu(r)	Absent or Leu-NH_2 Leu

[Formula V] comprises:

$$X\text{-}c[A^{-1}\text{-}B^{0}\text{-}C^{1}\text{-}D^{2}\text{-}E^{3}\text{-}F^{4}\text{-}G^{5}\text{-}H^{6}\text{-}I^{7}\text{-}J^{8}\text{-}K^{9}]$$

wherein X, A, B, C, D, E, F, G, H, I, J, and K are selected from the following or may be absent:

X	A -1	B 0	C 1	D 2	E 3	F 4	G 5	H 6	I 7	J 8	K 9
Absent or α-Aca 3,3Dp	Absent or Ava BAla DmK Glt Lys Suc	Absent or DArg DNik DPaF DPzK DPzO	Absent or Arg NiK PzO	Absent or Pro	Absent or Hyp	Absent or Gly	Absent or Add Aud Ava BAla DNMF Eac Igl Thi	Absent or DArg Ser Thr	Absent or DDab DDpr DF5F DIgl DLys DOrn DPaF Nig Pac Phe	Absent or DTrp F5F Lys Nc7G Oic PaF PFF Phe	Absent or Arg Leu NiK PaF 3Pal

[Formula V] also comprises:

$$X\text{-}c[A^{-1}\text{-}B^{0}\text{-}C^{1}\text{-}D^{2}\text{-}E^{3}\text{-}F^{4}\text{-}G^{5}\text{-}H^{6}\text{-}I^{7}\text{-}J^{8}]\text{-}K^{9}$$

[Formula V] also comprises:

$$X\text{-}c[A^{-1}\text{-}B^{0}\text{-}C^{1}\text{-}D^{2}\text{-}E^{3}\text{-}F^{4}\text{-}G^{5}\text{-}H^{6}\text{-}I^{7}]\text{-}J^{8}\text{-}K^{9}$$

wherein the cyclization is via a side chain functional group other than the C-terminal residue and the residues are as described in the immediately preceding table.

[Formula VI] comprises the following cyclic peptides:

B9458-2 DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-CpGΨ(CH_2N)Arg
|_____CO_____CH_2 _____/ \ CH_2CO_2H

B9462 DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-CpGΨ(CH_2N)Arg
|_____CO_____CH_2 _____/ \ CH_2CO_2H

ACA can also be those compounds in Table 4.

[Formula VII] comprises:

$[ACA]_1$-Eac-Eac-$[ACA]_2$

wherein [ACA] is defined by Formula I or the compounds in Table 4.

The *in vivo* inhibitory effects of antagonists may be studied using tumor-bearing nude mice. A tumor model employing nude mice orthotopically implanted with human lung cancer cells wherein the ACA is delivered by intratracheal instillation and aerosol inhalation may be used to evaluate the efficacy and feasibility of these antagonists as a means of treating human lung cancers. Control animals without tumor implantation may also be used to study the general side effects or cytotoxicity of the compounds. It is believed that aerosolized delivery or intratracheal instillation of the agents can produce effective dose accumulation in the area of lesion and reduce the overall systemic toxicity of the compounds in the animals more than when the compound is delivered by systemic administration.

The compounds may be administered topically, or by injection or infusion or as an oral suspension in an appropriate vehicle or as tablets, pills, capsules, caplets or the like, or preferably via intratracheal instillation or aerosol inhalation. The dosage and manner of administration will be defined by the application of the ACA and can be determined by routine methods of clinical testing to find the optimum dose. These doses are expected to be in the range of 0.001 mg/Kg to 100mg/Kg of active compound.

The compounds are composed of amino acids which may form salts due to their acidic or basic nature, and any pharmacologically acceptable salt derived from the compounds described in this invention such as hydrochlorides, acetates, phosphates, maleates, citrates, benzoates, salicylates, succinates, ascorbates and the like, including HCl, trifluoroacetic acid (TFA), and HOAc, are considered an extension of this invention. A common tactic in medicinal chemistry is to modify known drug substances which are peptide based to form esters or amides which exhibit greater bioavailability. Prodrugs derived from the compounds disclosed here are

therefore considered an obvious extension of this invention. Methods for designing and preparing prodrugs are described in detail in the medicinal chemical literature.

Structures and biological activities of peptides and peptide mimics related to bradykinin (BKR) are given in Table 1. Structures and biological activities of compounds not related to bradykinin (BKU) are given in Table 2. Structures and biological activities of cyclic peptides are given in Table 3. Structures of previously described known peptides which we have found to be active against cancers *in vivo* are included in Table 4. Actions of selected compounds on prostate cancer cell lines are given in Table 5. Abbreviations used are as defined in Table 6.

EXAMPLES

In general, Anti-bradykinin activity was determined by the classical guinea pig ileum assay and on Chinese hamster ovary (CHO) cells expressing cloned human bradykinin B2 receptors. Anti-tumor activity was determined on cultured human cancer cell lines using the standard tetrazolium (MTT) assay. No correlation between anti-bradykinin and cytolytic activity was found among the compounds, indicating that cells are not killed due to inhibition of an essential bradykinin function. Potent compounds were found to stimulate apoptosis in SCLC cells, probably by abnormal activation of the intracellular MEKK pathway.

EXAMPLE I – Synthesis of peptides

Peptides were synthesized using standard solid phase synthesis methods, well known in the art (Stewart and Young, *Solid Phase Peptide Synthesis*, Pierce Chemical Co., Rockford, IL, 1984) and were purified by HPLC and were characterized by amino acid analysis (AAA), thin layer chromatography (TLC) and laser desorption mass spectrometry (LDMS). Peptide amides were synthesized on methylbenzhydrylamine (MBHA) resin, which yields amides directly. Peptide methyl esters (OMe) were synthesized by reaction of peptides with 2,2-dimethoxypropane (Rachele, *J. Org. Chem.* 28: 2898, 1963). Cyclic peptides were prepared on resin or in solution with PyAOP and HOAt .

EXAMPLE II – Synthesis of non-peptides

Non-peptides were synthesized by standard organic chemistry procedures well known in the art. Compounds were purified by HPLC and were characterized by analytical HPLC, TLC, and LDMS.

EXAMPLE III – Synthesis of DDD and SUB dimers

Synthesis on resin: Neutralized peptide-resin (0.05mmole) was treated with 0.15 mmole diisopropylethyl amine (DIEA) and 0.026 mmole dodecanedioyl dichloride or suberoyl dichloride in 2.5 mL dichloromethane (DCM). The suspension was mixed for 5 h, washed with DCM and ethanol and dried. The peptide dimer was cleaved from the resin with HF, and the peptide was extracted and purified

Synthesis in solution: Carboxyl-derivatized amino acids or dipeptides were dissolved in dimethyl formamide (DMF) and treated with 10 equivalents of DIEA and 0.55 equivalent of dodecanedioyl dichloride or suberoyl dichloride overnight. The DMF was evaporated *in vacuo* and the resulting dimer was purified by HPLC.

EXAMPLE IV – Synthesis of EGS, DTP, SBEC and SUB dimers in solution

Dimerization in solution proceeded by reacting 1 equivalent of peptide monomer trifluoroacetate, an excess of DIEA and 0.55 equivalent of cross-linking reagent overnight in DMF. The cross-linking agents were purchased from Pierce (EGS dimer, ethylene glycol *bis*-(succinimidylsuccinate); DTP dimer, dithio*bis* (succinimidyl propionate); SBEC dimer, *bis*[(2(succinimidooxycarbonyloxy)ethyl]sulfone; SUB dimer, disuccinimidyl suberate).

EXAMPLE V – Synthesis of Boc-N-cycloheptylglycine (Nc7G)

N-Cycloheptylglycine was synthesized by reductive amination of cycloheptanone with glycine methyl ester following the procedure described in Gera *et al.*, *Immunopharmacology.* 33:174-177 (1996). The crude product was converted to the N-Boc derivative (mp, 89-90 °C).

EXAMPLE VI – Synthesis of TDIM dimers

Dimethyl tetradecyldiimidate was synthesized from tetradecanedinitrile by the method of De Abreu et al. (*Eur. J. Biochem.* 97: 379- 387, 1979. One equivalent of peptide TFA salt or other molecule having a free amino group was dissolved in DMF and stirred with 10 equivalents of DIEA and 0.7 equivalent of dimethyl tetradecyldiimidate dihydrochloride overnight at room temperature. DMF was evaporated *in vacuo* and the dimer was purified. SUIM dimers were prepared similarly, using dimethyl suberimidate.

EXAMPLE VII – Synthesis of B10238: F5C-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg (F5c-B9430)

B10238 was made by standard solid phase synthesis procedures, or by the acylation of B9430 with 2,3,4,5,6-pentafluorocinnamic acid in DMF, using BOP coupling in presence of excess DIEA. The product was purified by HPLC.

EXAMPLE VIII – Synthesis of M822: DDD-(DArg-F5F-Arg)$_2$

Following standard solid phase synthesis procedures, Boc-Arg(Tos) Merrifield synthesis resin was coupled with Boc-F5F, followed by coupling with Boc-DArg(Tos), using HATU as coupling agent. The peptide-resin was deprotected with TFA-DCM and neutralized with TEA. The peptide-resin was then treated with 0.55 equivalent of dodecanedioyl dichloride and 5 equivalents of DIEA in DCM overnight at room temperature. After washing and drying, the resin was cleaved with anhydrous HF, using standard conditions. The peptide was extracted from the resin with 90% acetic acid and purified by preparative HPLC.

EXAMPLE IX – Synthesis of M570 Hydrochloride: F5c-OC2Y-Atmp.HCl

4-Amino-2,2,6,6-tetramethylpiperidine (Aldrich) was coupled with Boc-(O-2,6-dichlorobenzyl)-tyrosine, using BOP in DMF solution. The Boc protecting group was removed by TFA and the product coupled with 2,3,4,5,6-pentafluorocinnamic acid in DMF, using BOP in the presence of excess DIEA at room temperature for 3 h. The DMF was removed *in vacuo*, the product was extracted into ethyl acetate and the solvent was evaporated. The residue was treated with 0.1 - 1.0 N HCl or 20% ethanolic HCl. The solvent was removed by evaporation *in vacuo* at room temperature. The residue was lyophilized from water-dioxane or crystallized from ethanol-ether.

EXAMPLE X – Synthesis of M630: Dmac-OC2Y-Matp.TFA

4-Methylamino-2,2,6,6-tetramethylpiperidine (Matp) was synthesized from 2,2,6,6-tetramethyl-4-piperidone (Aldrich) and methylamine by reductive amination with $NaCNBH_3$. The Matp was coupled with Boc-(O-2,6-dichlorobenzyl)-tyrosine, using BOP in DMF solution. The Boc protecting group was removed by TFA and the product was coupled with 4-(dimethylamino)cinnamic acid in DMF, using BOP in the presence of excess DIEA at room temperature for 3 h. The DMF was removed *in vacuo*. The product was extracted into ethyl acetate and the solvent was evaporated *in vacuo*. The crude product was purified by HPLC, giving the TFA salt. The Dmac-OCTY-Matp.TFA salt can be converted to its HCL salt as in Example IX above.

EXAMPLE XI – Synthesis of M638: DDD-(DArg-Igl-Arg-Matp)$_2$

In sequence, Boc-Arg(Tos), Boc-Igl and Boc-DArg(Tos) were coupled to 4-methylamino-2,2,6,6-tetramethylpiperidine (Matp), using BOP as coupling agent in DMF in the presence of excess DIEA at room temperature for 3-5 h. After removal of DMF *in vacuo*, the product was extracted into ethyl acetate. After evaporation of the solvent, the residue was treated with TFA-

DCM to remove the Boc group. TFA was removed *in vacuo*. The DArg(Tos)-Igl-Arg(Tos)-Matp.TFA was treated with dodecanedioyl dichloride (0.55 equiv) and DIEA (5 equiv) in DCM for 5 h. The protecting groups were cleaved by HF and the lyophilized product was purified by HPLC. The M638.TFA salt was converted to its HCl salt, using 0.1 - 1.0 N HCl or 20% ethanolic HCl as in Example IX above.

EXAMPLE XII – Synthesis of M590: Atmp-Igl-Pac-α-Sbl-Lys-B9430

In sequence, Boc-Igl, Boc-Pac and mono-methyl sebacate were coupled to 4-amino-2,2,6,6-tetramethylpiperidine (Atmp), using BOP coupling agent in DMF in presence of excess DIEA at room temperature for 3-5 h. DMF was removed *in vacuo* and the product was extracted into ethyl acetate. After evaporation of the solvent, the methyl ester was hydrolyzed in methanol by 1N NaOH. The crude product (0.025 mmol Atmp-Igl-Pac-Sbl) was coupled to the peptide resin (0.02 mmol Lys(2-ClZ)-DArg(Tos)-Arg(Tos)-Pro-Hyp-Gly-Igl-Ser(Bzl)-DIgl-Oic-Arg(Tos)-Merrifield resin) using BOP/DIEA activation in DMF. The heterodimer peptide was cleaved from the resin with HF, using standard conditions. The peptide was extracted from the resin with acetic acid and purified by preparative HPLC.

EXAMPLE XIII – Synthesis of M872: c[DArg-Arg-Eac-Ser-DF5F-Oic-Arg]

Following standard solid phase synthesis procedures, Boc-DArg(Tos) was coupled to Boc-Arg(Tos) Merrifield synthesis resin, followed in sequence by Boc-Arg(Tos), Boc-Oic, Boc-DF5F, Boc-Ser(Bzl), and Boc-Eac, using HATU as coupling agent. After deprotection with TFA-DCM, the resin was cleaved with anhydrous HF using standard conditions. The peptide was extracted from the resin with 0.1% TFA-H_2O/dioxane and lyophilized. The peptide trifluoroacetate was cyclized with three equivalents of PyAOP and HOAt and 20 equivalents of DIEA in DMF at a concentration of 10^{-3} M. After removal of the solvent under reduced pressure, the product was lyophilized from dioxane-H_2O and purified by HPLC.

EXAMPLE XIV – Synthesis of M678: (Dns-DArg-Igl-Arg)$_2$-DDA

In sequence, Boc-Arg(Tos), Boc-Igl and Boc-DArg(Tos) (2 equivalents) were coupled to 1,10-decanediamine using BOP as a coupling agent in DMF in presence of excess DIEA at room temperature for 3-5 h. DMF was removed *in vacuo* and the product was extracted into ethyl acetate. The solvent was evaporated *in vacuo* and the residue was treated with TFA/DCM to remove the Boc group. TFA was removed *in vacuo*, and the product was treated with dansyl chloride (2 equivalents) and an excess of DIEA in DCM for 5 h. The Tos groups were cleaved by HF and the crude product was purified by HPLC.

EXAMPLE XV – Synthesis of M290: BTAC-(2-Nal-Atmp)$_3$

The benzene-1,3,5-*tris*-carbamido-ε-caproic acid linker was made from 1,3,5-benzenetricarboxylic acid and N-Boc-ε-caproic acid methyl ester, using the BOP coupling method. The methyl ester was hydrolyzed in methanol by 1N NaOH. The product (1 equivalent BTAC) was coupled to 2-Nal-Atmp (3 equivalents) in DMF, using HATU as coupling agent. The solvent was removed *in vacuo*, and the residue was purified by HPLC. The BTAC-(2-Nal-Atmp)$_2$-OH was also isolated as a by-product.

EXAMPLE XVI – Synthesis of M1040: EDTA-(OC2Y-ATMP)$_4$

Boc-(O-2,6-dichlorobenzyl)-tyrosine was coupled with 4-amino-2,2,6,6-tetramethylpiperidine overnight in DMF, using BOP as coupling agent in the presence of DIEA. After removal of DMF *in vacuo*, the residue was extracted into ethyl acetate and treated with TFA/DCM to cleave the Boc group. The TFA/DCM was evaporated *in vacuo* and the product (OCTY-ATMP) was lyophilized from dioxane/water. Ethylenediaminetetraacetic acid (0.25 equivalent EDTA) was coupled with OC2Y-ATMP trifluoroacetate (1 equivalent) in DMF, using BOP as coupling agent in the presence of DIEA. The solvent was removed *in vacuo* and the residue was purified by HPLC.

EXAMPLE XVII - Assay of anti-bradykinin activity on guinea pig ileum

Male Hartley guinea pigs that had been deprived of food overnight were sacrificed, and sections of terminal ileum, 25 mm in length, were dissected, attached to tissue holders and immersed in 10 ml tissue baths containing Krebs' solution bubbled with $95\%O_2/5\%CO_2$. Tissues were placed under 1 g tension and incubated for 1 h equilibration. Concentration-effect curves were constructed to bradykinin in the absence and presence of new compounds. Bradykinin showed $pD_2 = 7.4$, and antagonist B9430 showed $pA_2 = 7.9$.

EXAMPLE XVIII - Assay of anti-bradykinin activity on cloned human B2 receptors

Chinese hamster ovary cells containing cloned and expressed human bradykinin B2 receptors were grown in cell cups of the Cytosensor microphysiometer in Ham's F-12 medium supplemented with sodium pyruvate and 10% FBS (Gibco 11765-054). For assay the cells were transferred to Ham's F-12 without bicarbonate or serum (Gibco 21700-075) and placed in the Cytosensor. Concentration-response curves were constructed to bradykinin in the presence or absence of new compounds. Bradykinin showed $pD_2 = 11$, and antagonist B9430 showed $pA_2 = 10.5$

EXAMPLE XIX - Colorimetric tetrazolium assay for cell survival

Cell growth and survival were measured by a rapid colorimetric assay based on the tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Mosmann, *J. Immunol. Methods* 65: 55-63, 1983, with minor modifications). Briefly, 1,000 normal lung fibroblasts or normal epithelial BEAS-2B cells, 1,000 or 5,000 viable non-SCLC cells or 10,000 viable SCLC cells were plated in 100 µL of growth medium in 96-well flat-bottomed microtiter plates. Cells were incubated overnight to allow recovery. Compounds to be tested were added to the cells in triplicate in a range of concentrations and the cells were incubated at 37°C, 5% CO_2, with 100% humidity. Control cells were treated in the same way without antagonists. All wells had a final volume of 200 µL. Plates were incubated for 4 days, allowing

sufficient time for cell replication and compound-induced cell death to occur. On day 5, 25 μL of a 2 mg/mL solution of MTT (Sigma) dissolved in RMPI-1640 was added to each well. The plate was incubated for 4 h at 37°C. The supernate was removed and the blue formazan complex was dissolved by adding 100 μL of 0.02 N HCl in 75% isopropanol to all wells. Absorbance was immediately determined using a scanning multiwell plate reader. B9870 caused 50% cell death at a concentration of 0.15 μM under these conditions.

EXAMPLE XX - Measurement of apoptosis in cultured cells

Apoptosis, also known as programmed cell death, is the phenomenon by which a cell dies following a series of gene-mediated events, in response to a wide range of intracellular and extracellular agents. Apoptosis, a counterpart of mitosis, plays an important role in the development and homeostasis of many organisms and tissues. It serves to regulate cell numbers, to shape developing organisms and as a defense against potentially harmful agents. Apoptosis is not the only mode of cell death. Necrosis is a type of cell death which is nonspecific and frequently occurs when cells are exposed to high doses of toxic agents. Such exposure usually results in the loss of ionic homeostasis. Unlike apoptosis, necrosis does not seem to be genetically influenced.

Apoptotic and necrotic cells have different appearances which allow them to be distinguished microscopically. Necrotic cells and their mitochondria swell, the cell membrane eventually ruptures, and internal organelles become distended. As a result of the membrane rupture, inflammation occurs in the surrounding tissue. In contrast, the nuclei of apoptotic cells become fragmented into several smaller nuclear bodies, which are quickly recognized by phagocytes and engulfed, and no inflammatory response occurs. Therefore, it is useful to develop chemotherapeutics which induce apoptosis, rather than necrosis, in order to avoid inflammation and the toxic agents which are often released from necrotic tumor cells.

We have used differential fluorescent dye uptake and cellular morphology to distinguish viable and dead cells with apoptotic and/or necrotic morphologies. We have used Rhodamine 123

to stain active mitochondria in viable cells, Hoechst 33324 to stain DNA in both viable and dead cells, and Propidium Iodide to stain DNA in dead cells. These cell subpopulations may be distinguished by the different manners in which they take up the fluorescent probes. The dead apoptotic and necrotic subpopulation, which has lost its membrane potential and organelle function, takes up Propidium Iodide and Hoechst 33324. Since the cells in this subpopulation are dead, the mitochondria are not active and thus there is little or no uptake of Rhodamine 123. Under the fluorescence microscope with a DAPI filter, nuclei in these cells appear pinkish in color due to the mixing of both Propidium Iodide and Hoechst 33324 dyes. Necrotic cells have intact nuclei while apoptotic cells have fragmented multi-nucleated bodies. In contrast, the viable apoptotic subpopulation has an intact membrane but inactive mitochondria. As a result, the fragmented multi-nucleated bodies (a hallmark of apoptotic cells) in these cells take up only Hoechst 33324, which gives them a blue appearance under the fluorescence microscope, but are unable to take up Propidium Iodide or Rhodamine 123. The subpopulation of viable cells has both intact cell membranes and active mitochondria. These cells take up both Hoechst 3324 and Rhodamine 123. Microscopically these cells appear to have single normal blue nuclei when examined with a DAPI filter and bright green mitochondria when examined with a FITC filter.

EXAMPLE XXI – Inhibition of tumor growth *in vivo* in nude mice

Representative peptide and non-peptide compounds having high *in vitro* cytotoxic activity were tested against implanted tumors *in vivo*. Athymic nude mice were implanted subcutaneously with either single cell suspensions (2 million SCLC cells or 1 million NSCLC cells) or with small fragments (3 x 3 mm) of tumors minced from previously grown nude mouse heterotransplants. On the seventh day after tumor implantation groups of 5 mice bearing implants were injected intraperitoneally daily with the compounds being tested at 1, 5, or 10 mg/kg/day; control animals were injected with an equal volume of isotonic saline. Tumor size was measured with a caliper three times per week. Tumor volume was calculated by the formula:

$$\text{Volume (cc)} = \pi \times (\text{length}) \times (\text{width})^2 / 6$$

Results of representative *in vivo* tests are given in Figs. 1-8. For comparison, bradykinin antagonist peptide dimers B9870 and B10054 caused marked inhibition of growth of the SCLC line SHP-77 at a dose of 5 mg/kg/day.

EXAMPLE XXII - Data

Examples of peptides and peptide mimics related to the C-terminal part of bradykinin antagonist peptides and their biological activities on cancer cells and bradykinin responses are given in Table 1.

Many compounds not directly related to the structure of bradykinin were synthesized and tested for anti-tumor and anti-bradykinin activity. These are listed in Table 2.

Cyclic peptides related to bradykinin and bradykinin mimics are reported in Table 3, along with their biological activity on cancer cells and anti-bradykinin activity.

Structures of previously described known peptides which have been found to be active against cancers *in vivo* are included in Table 4.

Cytotoxic activity *in vitro* of compounds M570 and M590 against various standard strains of prostate cancer is reported in Table 5.

Standard abbreviations were used for natural amino acids. For non-natural amino acids, derivatizing groups and other chemicals, the abbreviations listed in Table 6 are used.

Table 1. ACTIVITIES OF PEPTIDES RELATED TO BRADYKININ STRUCTURE

NUMBER	STRUCTURE	MTT[a]	GPI[b]	HUMAN[c]
BK[d]	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg			
B9430[d]	DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg	120	8.2	
B9870-2[d]	SUIM-(DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg)	0.15	8.4	
B8838	DArg-Arg-Pro-Hyp-Gly-CpG-Ser-DCpG-CpG-Arg	--	7.0	
B8840	DArg-Arg-Pro-Hyp-Gly-Phe-Ser-DCpG-CpG-Arg	--	6.8	
B8858	DArg-Arg-Pro-Hyp-Gly-Thi-Ser-CpG-DCpG-DArg	--	5.2	
B8994	DArg-Arg-Pro-MeP-Gly-CpG-Ser-DCpG-CpG-Arg	--	--	
B9074	Dhq-DArg-Arg-Pro-Hyp-Gly-CpG-Ser-DCpG-CpG-Arg	--	6.3	
B9126	Aaa-DArg-Arg-Pro-Hyp-Gly-(D,L)DMF-Ser-DTic-Oic-Arg	--	6.4	
B9126-2	Aaa-DArg-Arg-Pro-Hyp-Gly-(D,L)DMF-Ser-DTic-Oic-Arg	--	7.3	
B9224-2	Aca-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-(D,L)Igl-Oic-Arg	--	8.4	
B9882	α-Sub-Lys(εFlu)-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg	--	--	
	└DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg	--	--	
B9914	Oic-Arg	--	Wk	
B9916	DIgl-Oic-Arg	--	Wk	
B9490	Dcg-Digl-Oic-Arg	>60	Wk	
B9918	Ser-Digl-Oic-Arg	--	--	
B9920	Igl-Ser-DIgl-Oic-Arg	--	--	
B9922	Gly-Igl-Ser-DIgl-Oic-Arg	--	Wk	
B9924	Hyp-Gly-Igl-Ser-Digl-Oic-Arg	--	--	
B9926	Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg	--	--	
B9950	α-Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg | Sub-Arg-DNMF-DTrp-Phe-DTrp-Leu	8	--	
B9956	α-DDD-(Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg)$_2$	--	--	
B9960	DArg-Arg-Nig-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg	--	7.7	
B9966	DArg-Arg-NMF-Hyp-Gly-Thi-Ser-DIgl-Oic-Arg	--	6.9	
B10010	DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Nc7G-Arg	--	7.7	
B10014	DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Nc6G-Arg	--	7.6	
B10054	DDD-(Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg)$_2$	0.3	7.1	
B10062	DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg-NH_2	Inact	7.1	
B10082	SUIM-(DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg-NH_2)$_2$	0.7	7.2	
B10084	BApG-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg	>20	8.1	
B10088	DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg-Eac-Eac- DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg	4	7.1	

B10092	$(Gun)_2$-BApG-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg	>20	8.7	
B10098	$(DArg-Arg-Pro-Hyp)_2$-Dpr-Igl-Ser-DIgl-Oic-Arg	20	5.3	
B10100-2	TDIM-$(DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg)_2$	1	8.0	
B10100-1	Moti-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg	4	7.8	
B10104-2	TDIM-$(DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic-Arg)_2$	4	8.0	
B10104-3	Moti-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic-Arg	20	8.1	
B10160	Leu-DTrp-Phe-DTrp-DNMF-Eac_2-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg	10	6.3	
B10162	Leu-Leu-DTrp-Phe-DTrp-DNMF-Eac_2-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-Digl-Oic-Arg	7	6.3	
B10198-1	DDD-$(Eac-Arg-Digl-Oic-Arg)_2$	--	5.7	
B10198-2	DDD-$(Eac-Arg-Digl-Oic-Arg)_2$	15	--	
B10200	DDD-$(Eac-Eac-Arg-DIgl-Oic-Arg)_2$	16	5.8	
B10238	F5c-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg	150	8.1	
B10252	EGS-$(DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg)_2$	25	8.3	
B10282	Arg-Pro-Pro-Gly-Phe-Thr-DTic-Oic-Arg	--	7.3	
B10284	Arg-Pro-Pro-Gly-Phe-Thr-DTic-Oic-NH_2	--	7.7	
B10382	DArg-PzO-Pro-Hyp-Gly-Igl-Ser-DF5F-Oic-Arg	--	--	
B10384	DNiK-PzO-Pro-Hyp-Gly-Igl-Ser-DF5F-Oic-Arg	--	--	
B10386	DDD-$(DmK-PzO-Pro-Hyp-Gly-Igl-Ser-DF5F-Oic-Arg)_2$	--	--	
B10388	DNiK-PzO-Pro-Hyp-Gly-Igl-Ser-DF5F-Oic-Arg	--	--	
B10390	DNiK-PzO-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg	--	--	
B10392	DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-PFF-Arg	--	--	
B10394	F5c-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DF5F-PFF-Arg	--	--	
B10396	F5c-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-PFF-Arg	--	--	
M2	Dcg-D-2-Nal-Arg	--	--	
M10	Dcg-2Nal-Arg	--	4.8	--
M20	Gun-2-Nal-Arg	--	4.6	
M42	Gun-Eac-DIgl-Oic-Arg	--	5.0	
M68	Dcg-BtA-Arg	--	4.9	
M70	Dcg-Igl-Arg	--	4.8	
M78	Dcg-Apa-Arg	--	5.6	
M84	Dcg-Ile-Arg	--	5.1	
M86	Dcg-Ac6c-Arg	--	5.0	
M88	Gun-Ica-Arg	--	4.7	
M94	Dcg-Aic-Arg	--	Wk	
M96	Dcg-(D,L)Atc-Arg	--	4.7	
M118-1	Ac-PaF(Mcg)-Arg	--	4.9	
M118-3	Ac-PdF-Arg	--	5.4	
M124	Dcg-Gly-Cmp-Arg	--	4.7	
M128	Dcg-Gly-Oic-Arg	--	Wk	
M130	Dcg-F5F-Arg	20	4.8	
M132	F5bz-F5F-Arg	60	Wk	
M134	Dcg-Trx-Arg	--	4.9	

M142	Ac-PaF(Sin)-Arg	--	Wk	
M146-1	Ac-PaF(Mcg)-*p*-ABz-Arg	--	Inact	
M146-2	Ac-PaF(Dcg)-*p*-ABz-Arg	--	4.9	
M148	F5c-*p*-ABz-Arg	--	5.1	
M160	Ste-2-Nal-Arg	--	Wk	
M176	F5c-*p*ABz-2Nal-Arg	Inact	5.4	--
M196	F5c-Gly-*m*ABz-2Nal-Arg	Inact	5.1	--
M198	Ac-Pac-Gly-*m*-Abz-2-Nal-Arg	--	5.1	
M200-1	Mcg-Pac-Gly-*m*-ABz-2-Nal-Arg	--	Inact	
M200-2	Dcg-Pac-Gly-*m*-ABz-2-Nal-Arg	--	4.9	
M216	F5c-*p*-APa-Arg	>180	--	
M226	DDD-(Arg-DIgl-Oic-Arg)$_2$	35	5.7	Inact
M232-1	Dcg-Atpc-Arg	--	4.7	
M232-3	Dcg-2-Nal-Atpc-Arg	--	5.1	
M346	Dcg-*p*-Amb-Arg	--	4.6	
M348	F5c-*p*-Amb-Arg	--	4.7	
M352	F5c-*p*-Amb-APa-Arg	--	4.7	
M370	F5c-Arg	--	4.8	
M372	F5c-APb-Arg	--	4.6	
M374	Tfmc-Arg	--	4.6	
M380	F5c-Tyr-Arg	--	Inact	
M382	F5c-Tic-Arg	--	4.7	
M388	F5c-Lys{(CH$_3$)$_3$}-Arg	--	4.9	
M392	F5c-Ana-Arg	--	4.5	
M394	F5c-Bip-Arg	--	4.7	
M398	F5c-Pac-Arg	--	Inact	
M400	DDD-(*p*ABz-2Nal-Arg)$_2$	22	5.1	11.5
M406	Arg-Eac-DIgl-Ana-Arg	--	Inact	
M410	F5c-Phe-Arg	--	5.4	
M412	F5c-*m*-APa-Arg	--	5.8	
M416	F5c-3-Pal-Arg	--	Wk	
M420	F5c-hPhe-Arg	60	7.0	10.9
M424	F5c-Thi-Arg	--	4.6	
M426	F5c-Trp-Arg	--	Inact	
M442	F5c-Oic-Arg	--	--	
M446	F5c-2Nal-Arg	60	4.7	9
M450	F5c-2Nal-Arg-NH$_2$	26	4.9	Inact
M484	DDD-(Pac-2Nal-Arg)$_2$	25	Inact	Inact
M494	DDD-(Lys-Pac-Gly-*m*ABz-2Nal-NH$_2$)$_2$	33	5.1	Inact
M498	DDD-(Pac-2Nal-Arg-NH$_2$)$_2$	24	4.9	Inact
M500	DDD-(*p*ABz-2Nal-Arg-NH$_2$)$_2$	40	0	11.4
M504	DDD-(Pac-2Nal-DArg-NH$_2$)$_2$	11	5.4	Wk
M508	DDD-(DArg-2Nal-Arg)$_2$	23	Inact	--
M510	DDD-(DArg-2Nal-Arg-NH$_2$)$_2$	8	Inact	11
M512	F5c-OC2Y-Arg	70	5.7	11
M516	DDD-(DArg-Arg-Aud-Pac-2Nal-Arg)$_2$	1.4	0	Ag
M518	DDD-(DArg-OC2Y-Arg)$_2$	15	Wk	10
M520	F5c-OBS-Arg	Inact	6.1	7
M528	F5c-MBC-Arg	Inact	Inact	
M540	Pya-hPhe-Arg	>100	Wk	
M542	Dca-hPhe-Arg	80	Wk	

M550	F5c-OBT-Arg	80	Inact	Ag
M552	DDD-(p-ABz-hPhe-Arg)$_2$	Inact	Inact	
M554	DDD-(DArg-hPhe-Arg)$_2$	--	5.1	
M558	Dcg-hPhe-Arg	100	Wk	
M560	DDD-(DArg-hPhe-Arg-NH$_2$)$_2$	50	Wk	
M564	DDD-(DArg-OBS-Arg)$_2$	--	Wk	
M590	Atmp-Igl-Pac-α-Sbl-Lys-B9430	4.5	7.5	Inact
M598	DDD-(Arg-DIgl-Oic-Arg-OMe)$_2$	13	--	10
M600	α-DDD-(Lys-B9430-OMe)$_2$	1.2	6.4	Ag
M608	DDD-(Eac-Arg-DIgl-Oic-Arg-OMe)$_2$	13	--	Inact
M612	F5c-DArg-hPhe-Arg	Inact	Wk	
M676	DDD-(DArg-Arg-Eac-Ser-DF5F-Nc7G-Arg)$_2$	--	--	
M682	F5c-Lys(F5bz)-Arg	--	4.9	
M686	F5c-NMF-Arg	29	5.6	
M688	F5c-Dpr(Fbz)-Arg	--	Inact	
M690	F5c-Dpr(Paa)-Arg	--	4.8	
M692	DDD-(DArg-Arg-Aud-Pac-hPhe-Arg)$_2$	--	Wk	
M696	F5c-DArg-Eac-2Nal-Arg	Inact	5.1	--
M698	F5c-DArg-Arg-Aud-Pac-2Nal-Arg	7.1	Wk	--
M706	Cin-hPhe-Arg	Inact	Wk	
M708	Ppa-hPhe-Arg	Inact	5.8	
M710	DDD-(DArg-Arg-Aud-Pac-2Nal-DArg-NH$_2$)$_2$	1.7	Wk	
M714	F5c-PCF-Arg	Inact	Wk	
M718	F5c-PFF-Arg	9	5.1	
M720	F5c-PaF(Ppa)-Arg	Inact	4.8	
M726	D-Arg-Arg-Aud-PaF(F5c)-Arg	Inact	5.6	
M728	DDD-(DArg-Arg-Aud-PaF(F5c)-Arg)$_2$	4	5.3	
M730	F5c-DhPhe-Arg	--	4.7	
M732	F5c-PNF-Arg	--	4.9	
M734	DDD-(DArg-Arg-Aud-Pac-PaF(Fbz)-Arg)$_2$	1.8	5.3	
M738	F5c-DArg-Eac-hPhe-Arg	Inact	5.3	
M746	DDD-(Pac-hPhe-Arg)$_2$	Inact	Inact	
M752-2	Pac-hPhe-Arg	Inact	Wk	
M752-5	Aaa-Ser-Pac-hPhe-Arg	Inact	Wk	
M752-6	Aaa-Pac-hPhe-Arg	Inact	Wk	
M754	Aaa-DPhe-hPhe-Arg	Inact	4.6	
M756	DDD-(DPhe-hPhe-Arg)$_2$	18	5.2	
M758	Saa-hPhe-Arg	--	Wk	
M764	Aaa-DTic-hPhe-Arg	--	4.8	
M766	F5c-DArg-Arg-Aud-DTic-hPhe-Arg	--	5.1	
M770	DDD-(DArg-Arg-Aud-DTic-hPhe-Arg)$_2$	8	Inact	
M772	Aaa-DIgl-hPhe-Arg	--	4.9	
M774	F5c-DArg-Arg-Aud-DIgl-hPhe-Arg	8	Inact	
M776	DDD-(DIgl-hPhe-Arg)$_2$	30	5	
M778-1	Pcc-hPhe-Arg	--	Wk	
M780	Mca-hPhe-Arg	--	Wk	
M782	Cca-hPhe-Arg	--	Wk	
M784	Ac-OC2Y-Arg	Inact	Wk	
M786	DDD-(DArg-Arg-Aud-DIgl-hPhe-Arg)$_2$	3.2	Wk	
M788	F5c-DArg-Arg-Aud-DTic-Oic-Arg	9	5	
M790	DDD-(DArg-Arg-Aud-DTic-Oic-Arg)$_2$	1.7	Inact	

M792	F5c-DArg-Arg-Eac-Ser-DTic-Oic-Arg	>100	4.9
M794	DDD-(DArg-Arg-Eac-Ser-DTic-Oic-Arg)$_2$	21	Inact
M796	F5c-DArg-Arg-Eac-Ser-DF5F-Oic-Arg	31	6.5
M802	F5c-Lys-Ser-DF5F-Oic-Arg	Inact	6.3
M804	DDD-(DArg-Arg-Eac-Ser-DF5F-Oic-Arg)$_2$	7.3	7.7
M806	Ava-Igl-Ser-DF5F-Oic-Arg	Inact	5.6
M808	DDD-(Lys-Ser-DF5F-Oic-Arg)$_2$	30	6.9
M810	F5c-F5F-Arg	40	4.6
M812	F5c-PFF-Arg-NH_2	15	Wk
M814	Ppa-PFF-Arg	Inact	Wk
M816	Dpa-PFF-Arg	52	4.6
M818	DDD-(DArg-PFF-Arg-NH_2)$_2$	60	Wk
M820	DDD-(DArg-PFF-Arg)$_2$	43	Inact
M822	DDD-(DArg-F5F-Arg)$_2$	25	Mixed
M826	F5c-MFF-Arg	76	Inact
M828	F5c-3,4F2F-Arg	--	Wk
M838	F5c-DArg-Arg-Aud-DIgl-PFF-Arg	7.4	5.1
M842	DDD-(DArg-Arg-Aud-DIgl-PFF-Arg)$_2$	1.4	Inact
M844	DArg-Arg-Aud-DIgl-PFF-Arg	12	Wk
M846	DDD-(DArg-Arg-Aud-DF5F-Oic-Arg)$_2$	2	7.1
M852	F5c-DArg-Arg-Eac-Ser-DIgl-Oic-Arg	Inact	5.9
M854	DDD-(DArg-Arg-Eac-Ser-DIgl-Oic-Arg)$_2$	7.3	5.9
M856	F5c-DArg-Arg-Aud-Ser-DIgl-Oic-Arg	21	5.4
M858	DDD-(DArg-Arg-Aud-Ser-DIgl-Oic-Arg)$_2$	4	6.3
M860	F5c-DArg-Arg-Add-Ser-DIgl-Oic-Arg	6	5.4
M862	DDD-(DArg-Arg-Add-Ser-DIgl-Oic-Arg)$_2$	1.3	5.6
M864	DDD-(DArg-Arg-Add-Ser-DIgl-PFF-Arg)$_2$	1.8	Inact
M868	Ac-Darg-Arg-Aud-DF5F-Oic-Arg	55	6.5
M888	F5c-DArg-Arg-Aud-Ser-DF5F-Oic-Arg	12.5	6.6
M890	DDD-(DArg-Arg-Aud-Ser-DF5F-Oic-Arg)$_2$	1.7	5.5
M922	DDD-(DNiK-Arg-Eac-Ser-DF5F-Oic-Arg)$_2$	--	--
M926	ζ-SUB-(ApC-F5F-Arg)$_2$	Inact	--
M930	α-DDD-(ApC-F5F-Arg)$_2$	Inact	--
M932	DDD-(DArg-Arg-Eac-Ser-DIgl-PFF-Arg)$_2$	6.0	--
M936	DDD-(DNiK-PzO-Eac-Ser-DF5F-Oic-Arg)$_2$	--	--
M944	DDD-(DArg-Arg-Eac-Ser-DF5F-PFF-Arg)$_2$	6.7	--
M946	F5c- DArg-Arg-Eac-Ser-DF5F-PFF-Arg	--	--
M950	α-DDD-(K-DArg-Arg-Eac-Ser-DF5F-Oic-Arg)$_2$	6.7	--
M952	DDD-(DmK-DArg-Arg-Eac-Ser-DF5F-Oic-Arg)$_2$	--	--
M954	Aaa-DArg-Arg-Eac-Ser-DF5F-Oic-Arg	10	--
M956	Aaa-DArg-Arg-Aud-Ser-DF5F-Oic-Arg	14	--
M958	F5bz-DArg-Arg-Aud-Ser-DF5F-Oic-Arg	18	--
M960	Aca-DArg-Arg-Aud-Ser-DF5F-Oic-Arg	21	--
M964	33Dp-DArg-Arg-Aud-Ser-DF5F-Oic-Arg	4-8	--
M968	Dmac-DArg-Arg-Aud-Ser-DF5F-Oic-Arg	15	--
M972	F5pa-DArg-Arg-Aud-Ser-DF5F-Oic-Arg	--	--
M974	DDD-(PzO-F5F-Arg)$_2$	--	--
M976	DDD-(DNiK-F5F-Arg)$_2$	--	--
M978	DDD-(DPzK-F5F-Arg)$_2$	--	--

M980	DDD-(DPzO-F5F-Arg)$_2$	--	--
M1024	SUB-(DArg-Arg-Eac-Ser-DF5f-Nc7G-Arg)$_2$	14	7.2
M1026	DTP-(DArg-Arg-Eac-Ser-DF5F-Nc7G-Arg)$_2$	70	6.9
M1028	SBEC-(DArg-Arg-Eac-Ser-DF5F-Nc7G-Arg)$_2$	28	6.7
M1030	EGS-(DArg-Arg-Eac-Ser-DF5F-Nc7G-Arg)$_2$	51	7.0
M1034	DDD-(DArg-F5F-DArg-NH_2)$_2$	--	--
M1036	DDD-(DArg-F5F-DArg)$_2$	40	5.4
M1038	ε-SUB-(Lys-DArg-Arg-Eac-Ser-DF5F-Nc7G-Arg)$_2$	--	6.4
M1042	Aca-DArg-Arg-Eac-Ser-DF5F-Oic-Arg	--	--
M1044	Gun$_2$-BApg-DArg-Arg-Eac-Ser-DF5F-Oic-Arg	--	--
M1046	(F5c-DArg-Igl-Arg)$_2$-DDA	--	--

Footnotes:

[a] ED_{50} for killing of SCLC strain SHP-77 *in vitro*, μM.

[b] pA_2 for bradykinin antagonist activity on isolated guinea pig ileum. The pD_2 of bradykinin is 7.4 on ileum. Higher numbers indicate higher potency.

[c] pA_2 for bradykinin antagonist potency on cloned human B2 receptors, pM. The pD_2 for bradykinin is 11. Higher numbers indicate higher potency.

[d] Data included for comparison

Inact = inactive; Mixed = showing both agonist and antagonist activity; Wk = weak

Table 2. ACTIVITIES OF COMPOUNDS NOT RELATED TO BRADYKININ

NUMBER	STRUCTURE	MTT[a]	GPI[b]	HUMAN[c]
B9948	Arg-DNMF-DTrp-Phe-DTrp-Leu	2.8	Wk	
B10222	DNMF-DTrp-Phe-DTrp-LeuΨ(CH_2NH)Leu-NH_2	6.0	5.2	
B10224-1	α-DDD-(Lys-DNMF-DTrp-Phe-DTrp-LeuΨ(CH_2NH)Leu-NH_2)$_2$	13	--	
B10224-2	α-DDD-(Lys-DNMF-DTrp-Phe-DTrp-LeuΨ(CH_2NH)Leu-NH_2)$_2$	7	--	
B10228	DDD-(DNMF-DTrp-Phe-DTrp-LeuΨ(CH_2NH)Leu-NH_2)$_2$	40	Wk	
B10242	Arg-Pro-Lys-Pro-DTrp-Gln-DTrp-Phe-DTrp-LeuΨ(CH_2NH)Leu-NH_2	40	5.6	
B10244	DArg-Arg-Pro-Lys-Pro-DTrp-Gln-DTrp-Phe-	12	5.4	

	DTrp-LeuΨ(CH_2NH)Leu-NH_2			
B10246	DArg-Pro-Lys-Pro-DTrp-Gln-DTrp-Phe-DTrp-LeuΨ(CH_2NH)Leu-NH_2	12	6.1	
>278	F5c-Iqa-Atmp	9	5.3	--
M8	Gun-Eac-D2Nal-PgF	--	Inact	
M12	Dcg-Igl-Aqu	20	5.0	--
M18	Dcg-2Nal-Aqu	30	6.6	--
M26	Gun-2Nal-GaP	--	4.9	
M30	Dcg-2Nal-Apa	--	5.4	
M32	Gun-2Nal-Apa	--	4.8	
M36	Dcg-D2Nal-Apa	--	5.0	
M38	Gun-D2Nal-Apa	--	4.9	
M62	Dcg-2Nal-Ama	--	4.8	
M64	Dcg-2Nal-APa-Sud	--	Ag	
M72-1	Dcg-Igl-Apa	--	4.7	
M72-2	Dcg-Igl-APa(anisyl)	--	4.6	
M76	Dcg-2Nal-*m*ABz	--	4.9	
M92-1	Dcg-2Nal-mA$_2$Bz	--	5.0	
M92-2	Dcg-2Nal-mA$_2$Bz(Gun)	--	4.8	
M92-4	Dcg-2Nal-mA$_2$Bz(Dcg)	--	5.0	
M104	Dcg-2Nal-3Pal	--	4.9	
M112	Dcg-D2Nal-*m*ABz	--	5.1	
M120	Dcg-2Nal-*p*ABz	--	4.7	
M122-1	Mcg-APa-*m*ABz	--	5.0	
M122-2	Dcg-Apa-*m*ABz	--	4.6	
M136	Sin-F5F-3Pal	--	Inact	
M162	Dcg-2Nal-Asp	--	4.9	
M168-1	2Nap-PaF(Mcg)	--	4.8	
M168-2	2Nap-PaF(Dcg)	--	4.7	
M172	Inp-Dpr(Dcg-2Nal)	--	4.9	
M174	Dcg-Asp-Aqu	--	Inact	
M180	F5c-*p*ABz-2Nal	--	5.1	
M188B	Dcg-2Nal-Asp(Aqu)	--	5.5	
M202	F5c-Gly-*m*ABz-2Nal	--	5.1	
M204	Ac-Pac-Gly-*m*ABz-Nal	--	5.0	
M218	2Nal-Atmp	Inact	4.8	
M222	Dcg-2Nal-Atmp	15	6.8	Inact
M228-2	Dcg(Me)-2Nal-Atmp(Me)	15	7.6	--
M236	Dcg-Igl-Atmp	>50	4.7	--
M240	Dcg-F5f-Atmp	32	5.1	--
M244A	Dcg-2Nal-Atpm	12	5.0	
M244B	Dcg-2Nal-Atpc	Inact	4.9	
M246	Dcg-D2Nal-Atmp	>50	5.7	
M248	F5c-2Nal-Atmp	3.2	6.2	--
M250	Aca-2Nal-Atmp	--	5.2	
M252	Dhq-2Nal-Atmp	Inact	4.8	

M254	TDIM-(2Nal-Atmp)$_2$	5	5.1	--
M254-1	TDIM-(2Nal-Atmp)$_2$	5	5.1	
M254-2	TDIM-(2Nal-Atmp)$_2$	5	5.8	
M258	Dcg-Igl-Aptp	--	5.0	
M262	Dcg-D2Nal-Atmp	6	5.3	
M264	Dcg-Trp-Atmp	16	4.7	--
M266	Dcg-Apa-Atmp	42	4.8	--
M268	F5c-2Nal-Tpac	10	5.2	--
M270	Dcg-2Nal-Tpac	--	6.1	
M272	Dpa-2Nal-Atmp	9	5.2	--
M274	Sin-2Nal-Atmp	36	4.7	--
M276	Dca-2Nal-Atmp	4.6	5.3	--
M280	TDIM-(Igl-Atmp)2	6	5.2	--
M280-1	Ctim-Igl-Atmp	21	Wk	
M280-2	TDIM-(Igl-Atmp)$_2$	6	5.2	
M286	Dtp-(2Nal-Atmp)$_2$	24	5.1	--
M288	Boc-2Nal-Atmp	Inact	5.2	--
M288A	Boc-2Nal-Atmp	>85	5.2	
M290-1	Btac-(2Nal-Atmp)$_2$	>60	Wk	
M290-2	Btac-(2Nal-Atmp)$_3$	20	Wk	
M292	Pac-Igl-Atmp	40	Wk	
M294	DDD-(Pac-Igl-Atmp)$_2$	1.8	Inact	10.3
M296	Pya-Bip-Atmp	15	Wk	
M302	Atcp-2Nal-Atmp	3.5	5.2	
M304	TDIM-(2Nal-Dmm)$_2$	4.2	5.9	--
M306	Gbz-2Nal-Atmp	>100	--	
M308	Pac-2Nal-Atmp	>75	5.0	
M310	DDD-(Pac-2Nal-Atmp)$_2$	1.2	5.1	Inact
M312	Tfmc-2Nal-Atmp	3.2	5.5	--
M314	F5c-2Nal-Aqd	25	4.6	
M316	F5c-Tyr-Atmp	50	--	
M318	F5c-Tyr(Bzl)-Atmp	3.6	5.1	--
M320	F5c-Oic-Atmp	13	--	--
M322	F5c-Tic-Atmp	7.6	--	--
M324	Dmac-2Nal-Atmp	3	5.2	--
M336-1	Dcg-2Nal-Asp-(R,S)Aqu	--	5.1	
M336-2	Dcg-2Nal-Asp-(R,S)Aqu	--	5.4	
M340	Dcg-Pac-Gly-*m*ABz-2Nal	--	4.8	
M342	Dcg-2Nal-Asp-Atmp	--	5.4	
M350	Dcg-2Nal-Glu-Atmp	--	5.0	
M354	Dcg-2Nal-PgF	--	5.2	
M362	Dcg-*p*APa-Asp-Atmp	--	4.9	
M364	F5c-*p*APa-Asp-Atmp	--	4.7	
M368	Tfmc-*p*APa-Asp-Atmp	--	4.7	
M396	F5c-2Nal-Cys(SO_3H)-Atmp	--	5.0	
M408	Pya-2Nal-Cyh	22	4.6	11.5
M418	F5c-BtA-Atmp	3.8	7.0	10.3
M422	Pya-*p*ABz-2Nal	52	--	Inact
M428	Pya-Gly-*m*ABz-Aqd	>300	--	
M430	DDD-(BtA-Atmp)$_2$	18	5.3	11.3
M432	DDD-(2Nal-Asp-Atmp)$_2$	70	--	10
M436-1	TDIM-BtA-Atmp	8	4.8	

M436-2	TDIM-(BtA-Atmp)$_2$	4.5	5.8	Ag
M438	F5c-3Pal-Atmp	26	4.8	10
M440	Dcg-BtA-Atmp	30	--	
M448	Dmac-BtA-Atmp	2.7	5.7	Ag
M456	F5c-Cys(Meb)-Atmp	4.7	5.3	Inact
M460	DDD-(3Pal-Nal-Cyh)$_2$	15	Wk	
M466	F5c-2Nal-3Ampy	Inact	Wk	
M470	F5c-2Nal-Ampz	11	5.2	Inact
M472	Dmac-2Nal-Ampz	25	5.3	Inact
M474	Pya-2Nal-3Abza	35	Wk	Inact
M476	Tha-BtA-Atmp	15	4.6	Inact
M478	Dmac-2Nal-Thm	30	5.1	Inact
M480-1	HOOC-DDD-Pac-2Nal-Ampz	45	5.1	
M480-2	DDD-(Pac-2Nal-Ampz)$_2$	--	5.6	
M492	F5c-*m*ABz-2Nal-Ampz	45	5.1	Inact
M506	Mse-Pac-BtA-Atmp	11	4.9	--
M526	F5c-2Nal-Dmp	10	5.4	Inact
M536	F5c-2Nal-Dmab	4	Wk	Inact
M538	DDD-(Pac-2-Nal-Dmp)$_2$	>80	Wk	
M568	F5po-2Nal-Atmp	10	5.8	Ag
M570	F5c-OC2Y-Atmp	1.8	5.6	Ag
M572	Dca-2Nal-Acep	2.6	Wk	Wk
M574	Dns-Tyr(Bzl)Atmp	4.5	--	Inact
M582	Dmac-OC2Y-Atmp	3	5.4	9.5
M584-A	DDD-[DArg(Tos)-2Nal-Atmp]$_2$	5	Inact	10.3
M584-B	DDD-(DArg-2Nal-Atmp)$_2$	5	5.7	11.3
M586-A	Mse-Pac-Igl-Atmp	15	5.3	12
M586-B	Seb-Pac-Igl-Atmp	40	Wk	12.3
M588	α-DDD-(Lys-DArg-2Nal-Atmp)$_2$	9.4	Wk	10
M592	F5c-OC2Y-Matp	1.5	4.9	Ag
M594	F5c-MC2Y-Atmp	3.7	5.0	8
M594	F5c-MC2Y-Atmp	3.7	5.0	8
M596-A	DDD-[Arg(Tos)-2Nal-Atmp]$_2$	15	5.0	
M596-B	DDD-(Arg-2Nal-Atmp)$_2$	8.2	Wk	Inact
M602	Chc-OC2Y-Atmp	12	--	10.8
M604	Pac-2Nal-Ecap	43	4.5	
M606	DDD-(Pac-2Nal-Api)$_2$	30	5.0	10
M614	F5c-(N-Dmb)-Tyr(Bzl)-OMe	9.1	Wk	
M616	DDD-(Pac-1Nal-Atmp)$_2$	1.4	5.4	--
M618	F5c-DArg-2Nal-Arg-Matp	18	--	--
M620	DDD-(DArg-2Nal-Arg-Matp)$_2$	2.0	5.5	--
M622	F5c-OC2Y-Mapp	1.2	5.7	--
M624	Dns-OC2Y-Matp	1.4	5.1	--
M626	Pya-OC2Y-Matp	3.7	4.8	--
M628	Cin-OC2Y-Matp	1.6	5.2	--
M630	Dmac-OC2Y-Matp	1.6	5.0	--
M632	Atcp-OC2Y-Matp	1.4	5.4	--
M636	DDD-(DArg-Arg-Aud-Pac-2Nal-Atmp)$_2$	1.7	5.8	--
M638	DDD-(DArg-Igl-Arg-Matp)$_2$	0.6	Inact	
M640	DDD-(DArg-BtA-Arg-Matp)$_2$	3.0	5.9	
M648	F5c-PaF(Mes)-Atmp	Inact	5.0	
M650	Atcp-OC2Y-Mapp	3.7	--	--

M652	Ppa-OC2Y-Mapp	7.5	5.7	--
M654	Sul-Atmp	Inact	4.5	
M656	Sul-2Nal-Atmp	13	5.4	
M660	DDD-(His-1Nal-Atmp)$_2$	30	Wk	--
M662	F5c-tLeu-Atmp	Inact	5.2	--
M664	F5c-OCIY-Matp	1.2	5.0	
M666	Dns-OCIY-Matp	1.3	5.0	
M668	SBEC-(DArg-2Nal-Arg-Matp)$_2$	3.4	5.2	
M670	DTP-(DArg-Igl-Arg-Matp)$_2$	Inact	5.1	
M672	HDD-(DArg-Igl-Arg-Matp)$_2$	--	--	
M674	DDD-(DArg-F5F-Arg-Matp)$_2$	3.5	Wk	
M678	(Dns-DArg-Igl-Arg)$_2$-DDA	1.1	5.3	
M724	F5c-DArg-Aud-OC2Y-Gly-Atmp	12	5.4	
M744	DDD-(DArg-2Nal-Arg-Dmab)$_2$	3.4	5.3	
M798	F5c-OC2Y-Dmab	37	--	
M800	DDD-(DArg-OC2Y-Dmab)$_2$	27	5.3	
M832	F5c-PFF-Dmab	47	4.6	
M834	DDD-(DArg-PFF-Arg-Dpea)$_2$	1.6	5.3	
M848	DDD-(DArg-F5F-Arg-Dmab)$_2$	--	--	
M880	DDD-(DArg-F5F-Arg-Dpea)$_2$	--	--	
M886-1	DDD-DArg-PFF-Arg-NH$_2$ └DArg-PFF-Arg-Dpma	3.2	Wk	
M886-2	DDD-(DArg-PFF-Arg-Dpma)$_2$	--	Inact	
M892	DDD-(DArg-PFF-Arg-PFF-NH$_2$)$_2$	8.5	Wk	
M900	DDD-(DArg-F5F-Arg-PaF-NH$_2$)$_2$	6.3	--	
M916	F5c-DArg-PFF-Arg-PFF-NH$_2$	5.7	4.9	
M1032	DDD-(DArg-Igl-Mapp)$_2$	15	5.4	
M1040	EDTA-(OC2Y-Atmp)$_4$	0.73	--	

Footnotes:

[a]ED_{50} for killing of SCLC strain SHP-77 *in vitro*, μM.

[b]pA_2 for bradykinin antagonist activity on isolated guinea pig ileum. The pD_2 of bradykinin is 7.4 on ileum. Higher numbers indicate higher potency.

[c]pA_2 for bradykinin antagonist potency on cloned human B2 receptors, pM. The pD_2 for bradykinin is 11. Higher numbers indicate higher potency.

Ag = agonist; Inact = inactive; Wk = weak

Table 3. ACTIVITIES OF CYCLIC PEPTIDES

NUMBER	STRUCTURE	MTT[a]	GPI[b]
B9458-2	DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-CpGΨ(CH_2N)Arg |_____CO_____CH_2 _____/ \\ CH_2CO_2H	--	6.1
B9462	Darg-Arg-Pro-Hyp-Gly-Thi-Ser-Digl-CpGΨ(CH_2N)Arg |_____CO_____CH_2 _____/ \\ CH_2CO_2H	7.3	6.0
B10302	c[DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg]	Inact	5.2
B10304	Aca-c[DArg-Arg-Pro-Hyp-Gly-Thi-Ser-Nig-Oic-Arg]	Inact	6.4
B10306	c[Arg-DNMF-DTrp-Phe-DTrp-Leu]	Inact	Wk
B10312	α-DDD-(c[Lys-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DF5F-Oic-Arg])$_2$	3.8	--
M680	c[DArg-Arg-Eac-Ser-DF5F-Nc7G-Arg]	--	--
M824	c[Ava-Igl-Ser-DF5F-Oic-Arg]	Inact	5.2
M850	c[DArg-Arg-Aud-DIgl-PFF-Arg]	1.4	5.1
M868-2	c[DArg-Arg-Aud-DF5F-Oic-Arg]	9.2	6.1
M870	c[DArg-Arg-Add-DF5F-Oic-Arg]	5.5	5.3
M872	c[DArg-Arg-Eac-Ser-DF5F-Oic-Arg]	2.2	Inact
M874	c[DArg-Arg-Add-Ser-DF5F-Oic-Arg]	11	5.0
M876	c[DArg-Arg-Aud-Ser-DF5F-Oic-Arg]	22.5	5.4
M878	c[DArg-Arg Add-DIgl-PFF-Arg]	7	Wk
M882	c[DArg-Arg-Add-Ser-DIgl-PFF-Arg]	4.5	Inact
M896	c[DArg-Arg-Eac-DIgl-PFF-Arg]	65	Wk
M902	c[DArg-Arg-Ava-Ser-DIgl-PFF-Arg]	30	5.5
M906	c[DArg-Arg-Eac-DF5F-Oic-Arg]	45	Wk
M908	c[DArg-Arg-Ava-Ser-DF5F-Oic-Arg]	40	4.9
M910	c[Bala-DArg-Arg-Eac-Ser-DF5F-Oic-Arg]	42	5.2
M924	c[Suc-DArg-Arg-Eac-Ser-DIgl-PaF]-Arg	37	Wk
M934	c[DNiK-Arg-Eac-Ser-DF5F-Oic-Arg]	--	--
M940	c[DNiK-PzO-Eac-Ser-DF5F-Oic-Arg]	--	--
M986	c[Add-DArg-F_5F-Arg]	--	--

Footnotes:

[a]ED_{50} for killing of SCLC strain SHP-77 *in vitro*, μM.

[b]pA_2 for bradykinin antagonist activity on isolated guinea pig ileum. The pD_2 of bradykinin is 7.4 on ileum. Higher numbers indicate higher potency.

Inact = inactive; Wk = weak

Table 4. PREVIOUSLY DESCRIBED KNOWN PEPTIDES THAT NEWLY SHOW *IN VIVO* ANTI-CANCER ACTIVITY

NUMBER	STRUCTURE
B9430	DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg
B9330	DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Nig-Arg
B10044	DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DF5F-Oic-Arg
B10050	Lys-Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DTic-ChG
B10206	DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DF5F-Nc7G-Arg
B10288	DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg

These compounds showed anti-tumor activity *in vivo* when tested by the procedure of Example XXI.

TABLE 5. CYTOTOXICITY *IN VITRO* AGAINST STRAINS OF PROSTATE CANCER

COMPOUND NUMBER	PROSTATE CANCER CELL LINE					SCLC
	DU14	TSU	LNCa	PC-3	PPC1	SHP-77
B9870	0.08	6.5	3.7	3.2	4.3	0.15
M570	1.2	2.8	3.0	1.6	3.0	1.8
M590	0.01	7.0	7.0	6.3	12	4.5

Numbers are ED_{50} (μM) for cytotoxic activity. Activity against SCLC strain SHP-77 is included for comparison.

TABLE 6. ABBREVIATIONS USED FOR COMPOUNDS

B9430 = DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg
B9870 = SUIM-(DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg)$_2$
Aaa = 1-Adamantaneacetyl
AAA = amino acid analysis
ABz = Aminobenzoic acid
ABza = Aminobenzyl alcohol
Ac = Acetyl
Ac3c = 1-Amino-1-cyclopropanecarboxylic acid
Ac5c = 1-Amino-1-cyclopentanecarboxylic acid ("cyclo-Leu")
Ac6c = 1-Aminocyclohexanecarboxylic acid
Aca = 1-Adamantanecarboxyl
Acep = 4-Amino-1-carbethoxymethyl-2,2,6,6-tetramethylpiperidine
ADA = 1,3-Adamantanediacetyl

Add = 12-Aminododecanoic acid
Aib = α-Aminoisobutyric acid
Aic = 2-Aminoindane-2-carboxylic acid
AlG = α-Allylglycine (2-amino-4-pentenoic acid)
Ama = Aminomethylanthranilic acid
Amb = Aminomethylbenzoic acid
Ampy = 3-Aminomethylpyridine
Ampz = 1-Amino-4-methylpiperazine
Ana = Anthranilic acid
APa = *p*-Aminophenylacetic acid
APb = *p*-Aminophenylbutyric acid
ApC = S-3-Aminopropylcysteine
Api = 4-Aminopiperidine
Apmp = 4-Amino-1,2,2,6,6-pentamethylpiperidine
Aptp = 4-Amino-1-phenylmethyl-2,2,6,6-tetramethylpiperidine
Aqd = 4-Aminoquinaldine
Aqu = 3-Aminoquinuclidine
Arg(NO_2) = Arginine(Nitro)
Atc = 2-Aminotetralin-2-carboxylic acid
Atcp = 4-Amino-3,5,6-trichloropicolinic acid
Atmp = 4-Amino-2,2,6,6-tetramethylpiperidine
AtmpO = 4-Amino-2,2,6,6-tetramethylpiperidinyloxy
Atpc = 4-Amino-2,2,6,6-tetramethyl-4-piperidinecarboxylic acid
Atpm = 4-Amino-4-methoxycarbonyl-2,2,6,6-Tetramethylpiperidine
(4-Amino-2,2,6,6-tetramethyl-4-piperidinecarboxylic acid methyl ester)
Aud = 11-Aminoundecanoic acid
Ava = 5-Aminovaleric acid
Azt = Azetidine-2-carboxylic acid
BAla = β-Alanine
BApG = N,N-*bis*(3-aminopropyl)-glycine
BAPTA = 1,2-*bis*(2-Aminophenoxy)ethane-N,N,N',N'-tetraacetyl
Bip = Biphenylalanine
Boc = (*tert*-Butoxycarbonyl); [(1,1-dimethylethoxy)carbonyl]
BOP = Benzotriazoyloxytris(dimethylamino)phosphonium hexafluorophosphate
BPHD = N,N'-*bis*(2,2,6,6-tetramethyl-4-piperidinyl)-1,6-hexanediamine
BSH = 1,6-Bissuccinimidohexane
BtA = 3-Benzothienylalanine
BTAC = Benzene-1,3,5-*tris*-carboxamido-6-caproyl
BTC = 1,3,5-Benzenetricarboxyl

Bz = Benzoyl
Bzl = Benzyl
CAcH = *cis*-2-Amino-1-cyclohexanecarboxylic acid
Cca: 2-Chlorocinnamic acid
CDF = *p*-Chloro-D-phenylalanine
ChA = α-Cyclohexylalanine
Chc = α-Cyano-4-hydroxycinnamoyl
ChG = α-Cyclohexylglycine
CHO = Chinese hamster ovary
CHTC = 1,3,5-Cyclohexanetricarboxyl
CHyp = *cis*-4-Hydroxy-proline
Cin = Cinnamoyl
CMeb = S-(4-Methylbenzyl cysteine
CmF = (Z) *p*-Chloro-2,3-methanophenylalanine
Cmp = 4-Carboxymethylpiperazine
CpA = α-Cyclopropylalanine
CpG = α-Cyclopentylglycine
CpGΨ(CH_2N)Arg = CpG pseudo(CH_2NH) Arg
CPTA = *trans*-1,2-Diaminocyclohexane- N,N,N',N'-tetraacetyl
CTAC = Cyclohexane-1,3,5-*tris*-carbamido-ε-caproyl
Ctim = 13-Carboxytridecanimidyl
Cyh = Cyclohexylamine
Dabz = Diaminobenzoic acid
DArg(NO_2) = Nitro-Arginine
Dca = Dicyclohexylacetyl
Dcg = N,N'-Dicyclohexylguanidyl
DCM = Dichloromethane
DDA = 1,10-Decanediamine
DDD = Dodecanedioyl
DDS = 2-Dodecen-1-ylsuccinyl
DEA = N,N'-Diethylethylenediamine
DhP = 3,4-Dehydroproline
Dhq = 2,3-Dehydroquinuclidine-3-carboxyl
DIC = Decahydroisoquinoline-3-carboxylic acid
DIEA = Diisopropylethylamine
Dmab = 4-Dimethylaminobenzylamine
Dmac = 4-Dimethylaminocinnamyoyl
Dmb = 4-(Dimethylamino)benzyl
DmF = 2,4-Dimethylphenylalanine
DMF = Dimethyl formamide
DmK = ε-Dimethyllysine

Dmm = 2,6-Dimethylmorpholine
Dmp = 3-Dimethylaminopropylamine
DmtP = 5,5-Dimethyl-4-thiaproline
Dns = Dansyl (5-dimethylamino-1-naphthalenesulfonyl)
22Dp = 2,2-Diphenylpropionyl
33Dp = 3,3-Diphenylpropionyl
Dpa = Diphenylacetyl
Dpea = Diphenylethylamine
Dpma = Diphenylmethylamine
Dpr = 2,3-Diaminopropionic acid
DTP = Dithiobis-propionyl
DTPA = Diethylenetriaminepentaacetyl
Eac = ε-Aminocaproic acid
Ecap = N-Ethoxycarbonyl-4-amino-piperidine (Ethyl 4-amino-1-piperidinecarboxylate)
EDA = 4,4'-Ethylenedianiline
EDP = 4,4'-Ethylenedipiperidine
EDTA = Ethylenediaminetetraacetyl
EDTP = Ethylenediaminetetrapropionic acid
EGS = Ethylene glycol-*bis*-succinyl
EGTA = Ethylene glycol-*bis*(β-aminoethyl ether)- N,N,N',N'-tetraacetyl
EOPC = 1,1'-Ethylenebis(5-oxo-3-pyrrolidinecarboxyl)
ETTA = 2,2'2'',2'''-[Ethanediylidenetetrakis(thio)tetrakisacetyl
F2F = Difluorophenylalanine
F5bz = Pentafluorobenzoyl
F5c = 2,3,4,5,6-Pentafluorocinnamoyl
F5F = Pentafluorophenylalanine
F5pa = 2,3,4,5,6-Pentafluorophenylacetyl
F5po = 2,3,4,5,6-Pentafluorophenoxyacetyl
Fbz = *para*-Fluorobenzoyl
Flu = Fluorescein thiourea
Gaa = Guanidinoacetyl
GaP = 2-Guanidyl-3-(4-aminophenyl)propionic acid
Gbz = 4-Guanidinobenzoyl
Glt = Glutaryl
Gun = Guanidyl
HATU = O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HbQ = 4-Hydroxybutylglutamine
HDA = 1,6-Hexanediamine
HDD = Hexadecanedioyl

HF = Hydrogen fluoride
HFG = Hexafluoroglutaroyl
HiG = Hexahydro-2-indanylglycine
HOAt = 1-Hydroxy-7-azabenzotriazole
hPhe = Homo-phenylalanine
HPLC = high performance liquid chromatography
Hxa = Hexanoic acid
Hyp = *trans*-4-Hydroxyproline
Ica = Indoline-2-carboxylic acid
Igl = α-2-Indanylglycine
Ing = α-1-Indanylglycine
Inp = Isonipecotic acid
Iq2a = 6,7-Dimethoxy-3,4-dihydro-1-isoquinolineacetic Acid
Iq4a = 6,7-Dimethoxy-1,2,3,4-tetrahydro-1-isoquinolineacetic acid
Lau = Lauroyl
Leu(r)Leu = Leu-pseudo(CH_2NH)Leu
LeuΨ(CH_2NH)Leu = Leu-pseudo(CH_2NH)Leu
LDMS = laser desorption mass spectrometry
mA_2Bz = 3,5-Diaminobenzoic acid
MaG = α-Methallylglycine (2-amino-3-methyl-4-pentenoic acid)
Mapp = 4-(Methylamino)-1,2,2,6,6-pentamethylpiperidine
Matp = 4-(Methylamino)-2,2,6,6-tetramethylpiperidine
MatpO = 4-(N-methylamino)-2,2,6,6-tetramethylpiperidinyloxy
MBC = S-(4-methylbenzylcysteine
MBHA = Methylbenzhydrylamine
MC2Y = N-Methyl-O-2,6-dichlorobenzyl-tyrosine
Mca = 2-Methylcinnamic acid
Mcg = Monocyclohexylguanidyl
Meb = Methylbenzyl
MeP = 2,4-Methanoproline
Mes = Methanesulfonyl
MFE = (E)-2,3-Methanophenylalanine
MFF = *meta*-Fluorophenylalanine
Mosi = Methoxy-suberimido
Moti = 14-Methoxytetradecanediimidoyl
Mse = Methoxysebacyl
MTT = (3-(4,5)-Dimethyltriazol-2-yl)-2,5-diphenyl tetrazolium bromide
Nal = β-Naphthylalanine
Nap = Naphthoyl
Nba = Norbornane-2-acetyl
Nbc = Norbornenedicarboxyl

Nbi = Norbornenedicarboximide
Nbn = 2-Aminonorbornane-2-carboxylic acid
Nc5G = N-Cyclopentylglycine
Nc6G = N-Cyclohexylglycine
Nc7G = N-Cycloheptylglycine
Nc8G = N-Cyclooctylglycine
Nig = N-2-Indanylglycine
NiK = ε-Nicotinoyllysine
NMF = N-Methylphenylalanine
NSCLC = non-small cell carcinoma
OBS = O-Benzylserine
OBT = O-Benzylthreonine
OBY = O-Benzyltyrosine
OC2Y = O-2,6-Dichlorobenzyltyrosine
OCIY = O-2,6-Dichlorobenzyl-3,5-diiodotyrosine
Oct = Octanoyl
Oic = Octahydroindole-2-carboxylic acid
OMe = O-Methyl
OMY = O-Methyltyrosine
OSY = Tyrosine O-sulfate ester
Paa = Phenylacetyl
Pac = 4-Aminocinnamic acid
PaF = *p*-Aminophenylalanine
Pal = β-Pyridylalanine
Pba = Phenylbutyryl
Pcc = *trans*-2-Phenyl-1-cyclopropanecarboxylic acid
PCF = *p*-Chlorophenylalanine
Pcpa = α-Phenylcyclopentaneacetyl
PdF = *p*-Dicyclohexylguanidylphenylalanine
PFF = p-Fluorophenylalanine
PFS = Perfluorosuberoyl
PgF = *p*-Guanidinophenylalanine
PheOL = Phenylalaninol
PhG = Phenylglycine
Pip = Pipecolic acid ("homo-Pro")
PipA = β-3-Piperidylalanine
PNF = *p*-Nitrophenylalanine
Ppa = Phenylpropionyl
Pya = *trans*-3-(3-Pyridyl)acryloyl
PyAOP = 7-Azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate

PzK = ε-Pyrazinoyllysine

PzO = 4-Pyrazinoylornithine 4-(2-pyrazin carboxyl) orn

Saa = *trans*-Styrylacetic acid

SBEC = Sulfo-*bis*-ethoxycarbonyl

Sbl = Sebacoyl

SCLC = small cell lung carcinoma

Seb = Sebacyl

Sin = Sinapinyl (3,5-dimethoxy-4-hydroxycinnamoyl-)

Ste = Stearoyl

Sua = Sulfanilamide (4-Aminobenzenesulfonamide)

SUB = Suberyl

Suc = Succinyl

Sud = Sulfadiazine

SUIM = Suberimidyl

Sul = Sulindac

Tba = *t*-Butyl-acetyl

TDIM = Tetradecanediimidyl

TEA = Triethylamine

TFA = Trifluoroacetic acid

Tfmc = *trans*-4-(Trifluoromethyl)cinnamoyl

Tha = 3-(2-Thienyl)acryloyl

Thi = β-2-Thienylalanine

Thm = Thiomorpholine

Thz = Thiazolidine-4-carboxylic acid (4-thiaproline)

Tic = 1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid

TLC = thin layer chromatography

TLeu = *tert*-Leucine

TMF = 2,4,6-Trimethylphenylalanine

Tos = *p*-Toluenesulfonyl

Tpac = 2,2,5,5-Tetramethyl-3-(aminoethyl)-pyrroline-3-carboxamide

TREN = *tris*(2-Aminoethyl)amine

Trx = Tranexamic acid (*trans*-4-((Aminomethyl))cyclohexanecarboxylic acid)